PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project	
Monitor and evaluate genetic characteristics of supplemented salmon and steelhead	
BPA project number	**8909600**
Contract renewal date (mm/yyyy)	**9/2000**
Multiple actions? (indicate Yes or No)	**No**
Business name of agency, institution or organization requesting funding	
National Marine Fisheries Service, Conservation Biology Division	
Business acronym (if appropriate)	**NMFS**
Proposal contact person or principal investigator:	
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NPPC Program Measure Number(s) which this project addresses	
4.1A.2, 4.1D, 7.2A.1, 7.2A.2, 7.2A.6, 7.3B.2 7.4D.1, 7.4L.1, 7.5B.1, 8.4A.1	
FWS/NMFS Biological Opinion Number(s) which this project addresses	
Conservation Recommendation #3 from the "Biological Opinion for 1995 to 1998 Hatchery Operations in the Columbia River Basin" (April 5, 1995) stipulates that "The action agencies should conduct monitoring and evaluation studies for hatchery programs. This should assist NMFS in evaluating the effects of hatchery programs on listed and unlisted natural fish." Consultation #383 - Biological Opinion on 1995-1998 Hatchery Operations in the Columbia River Basin. Issued 4/5/95 Expires 12/31/98. This BO addresses Lookingglass straying issue in Grande Ronde basin. Direct take permits (and associated Biological Opinions) that call for genetic monitoring of populations included in this study: Permit 847--expired June 30, 1998. Will be replaced by Permit 1128. Comment Period ended 4/23/98. Waiting for Section 7 consultation. Permit 919--expires December 31, 1998. Will be replaced by Permit 1179. Comment Period ends 12/17/98. Permit 921--expires December 31, 1998. Will be replaced by Permit 1179. Comment Period	

ends 12/17/98.

Permit 1011--expires December 31, 2000. Currently processing Mod 2. Comment Period ended 5/26/98. Waiting for Section 7 consultation.

Other planning document references

NMFS *Snake River Salmon Recovery Plan*: Tasks 4.1b, 4.3a; priority 1. *Wy Kan Ush Me Wa Kush Wit:* Monitoring, Types of Monitoring, level 2., Implementation and Coordination, benefits 2., 4.; Hypothesis 11, Stock-specific Harvest Management Concerns, Recommended Actions/Tests, points 1 and 2.

Short description

Monitor changes over time in genetic characteristics of hatchery, natural (supplemented), and wild (unsupplemented) populations of Snake River spring/summer chinook salmon and steelhead. Estimate reproductive success. Use results to help evaluate effectiveness of supplementation.

Target species

Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*)

Section 2. Sorting and evaluation

Subbasin

Steelhead: Tucannon, Grande Ronde, Imnaha, Clearwater
Spring/summer chinook salmon: Grande Ronde, Imnaha, SF Salmon, MF Salmon, upper Salmon

Evaluation Process Sort

CBFWA caucus		CBFWA eval. process		ISRP project type	
X one or more caucus		If your project fits either of these processes, X one or both		X one or more categories	
X	Anadromous fish	X	Multi-year (milestone-based evaluation)		Watershed councils/model watersheds
	Resident Fish		Watershed project eval.	X	Information dissemination
	Wildlife				Operation & maintenance
					New construction
				X	Research & monitoring
					Implementation & mgmt
					Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description
	N/A

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
92-26-1 ODFW	Early life history Grande Ronde chinook	We coordinate genetic sampling with sampling from this study.
90-052 NBS	Performance/stock productivity impacts of supplementation	We have shared steelhead samples from this study and we provide NBS results of our genetic analyses for some of their study streams.
91-073 & 89-098 IDFG	Idaho natural production and evaluation, Intensive monitoring subproject; ISS	We coordinate genetic sampling with sampling from these studies.
9005500 IDFG	Steelhead Supplementation Studies In Idaho Rivers	We coordinate genetic sampling with collections for this program.
9604400 ODFW/ NPT	Grande Ronde sp. chinook captive broodstock program	We coordinate genetic sampling with collections for this program.
9801001 LSRCP/ ODFW/ NPT	Grande Ronde sp. chinook captive broodstock O&M M&E	We coordinate genetic sampling with collections for this program.
8805301 NPT	NE Oregon outplanting facilities plan	Sampling will be coordinated with this program.
8805305 ODFW	NE Oregon outplanting facilities plan	Sampling will be coordinated with this program.
8805300 ODFW/ NPT	NE Oregon sp. chinook hatchery planning	We coordinate genetic sampling with this program.
8712700 NPT	Smolt monitoring by Fish Passage Center	Sampling will be coordinated with this program.
9606700 NMFS	Manchester captive broodstock O&M	We coordinate genetic sampling with this program.

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
1989-	Tissue samples taken for genetic monitoring	Essentially all samples collected

1998	and logged into the collection at NWFSC represent a major component of the largest tissue repository available for Pacific salmon (>18,000 samples)	through 1997 have been analyzed for allozyme variation. Over 5500 samples have been DNA-extracted and genotyped, and a variety of tissue samples have been made available to collaborators and comanaging agencies.
1991-1998	High levels of genetic variability documented within and among Snake River chinook salmon and steelhead populations. This variability shown to be stable through time.	The geographic distribution of genetic variation revealed in this study was used extensively in the status reviews for both of these species (Busby et al. 1996; Myers et al. 1998).
1991-1997	Allozyme data supported distinctiveness of Dworshak Hatchery steelhead. Distinctiveness appeared to be ancestral.	Provided an improved understanding of the genetic relationship between resident fish in the NF Clearwater and the hatchery population
1991-1996	Estimation of N_m and the critical ratio of N_b/N	Results from this study provide the most comprehensive data available for salmon for these important parameter estimates
1996	Allozyme data played a critical role in the US v. Oregon dispute resolution	Data were provided to the Independent Scientific Review Panel for their own analyses
1995-1998	New restriction site markers developed for nuclear DNA loci. >95 primer pairs have been made for introns, 3' & 5' untranslated regions, random clones, and other noncoding sequences.	Used to describe genetic structure of selected Snake River populations. Results published in peer reviewed literature (3 papers). Continued efforts ongoing. Primers distributed to other laboratories.
1995-1998	Groups of microsatellite markers (multiplex sets) developed and implemented in both chinook salmon and steelhead, permitting rapid and efficient genotyping. >90 microsatellite primer pairs made.	Multiplex sets used to collect data for multiple studies related to genetic monitoring. Substantial reductions in time effort and expense associated with genotyping.
1996	DNA markers (nonlethally analyzed) provided information on the relative distinctiveness of NE Oregon spring chinook salmon captive brood stock collections as compared to the Rapid River stock spawned at Lookingglass hatchery	Information was requested by ODFW at the outset of the captive brood program (we were not permitted at the time to offer results directly to the Independent Science Panel, unless specifically requested).
1998	DNA data helped evaluate potential distinctiveness of marked and unmarked fish returning to the trap at the Rapid River Hatchery	Interagency memo provided to Sharon Kiefer and Rick Lowell, IDFG with copies distributed to other comanagers.
1998	Developed an analytical solution for the Phelps/Allendorf effect, a common sampling	Published in peer reviewed literature

	problem associated with the collection of juveniles when population sizes are small	
1996-1998	Technological developments in the rapid assay of single nucleotide polymorphisms (SNPs)	Results published in peer reviewed literature (2 papers). Continued efforts ongoing.
1998	Development of DNA extraction and genotyping of historic scale samples	Methodological experiments completed. Manuscript in preparation.

Objectives and tasks

Obj 1,2,3	**Objective**	**Task a,b,c**	**Task**
1	Collect samples	a	Consult comanagers and conduct preseason evaluations of previous year escapements to identify optimal sampling strategy
		b	Coordinate sampling efforts to maximum extent possible with other ongoing projects
		c	Collect samples from hatchery, natural, and wild populations
2	Conduct genetic analyses	a	Perform allozyme and DNA analyses
		b	Perform quality control tests on preliminary data
3	Measure levels of genetic variability in each population	a	Quantify percent polymorphic loci, heterozygosity, number of alleles per locus
		b	Compare values in hatchery, natural, and wild populations
		c	Evaluate pattern of change in genetic variability over time
4	Estimate effective population size (N_e) and the ratio N_e/N for each population	a	Compute F, a measure of temporal change in allele frequency
		b	Compute r^2, a measure of gametic disequilibrium
		c	Use temporal and disequilibrium methods to obtain a combined estimate of Ne for each population
		d	Estimate total population size (N) based on redd counts, spawner surveys, or population enumeration
		e	Compute ratio N_e/N
5	Evaluate population genetic structure of natural and wild	a	Compute indices of genetic differentiation among natural and wild

Obj 1,2,3	Objective populations	Task a,b,c	Task populations
		b	Perform hierarchical gene diversity analyses to partition genetic differences into various components
		c	Estimate levels of gene flow among populations based on genetic data
6	Evaluate genetic effects of supplementation on target and non-target populations	a	Compute indices of genetic differentiation between hatchery and natural and hatchery and wild populations
		b	Compare patterns of genetic change over time in hatchery populations with those in natural and wild populations
		c	Compare recent genetic data for Grande Ronde populations with historic (pre-supplementation) data obtained from DNA analysis of archived scales
		d	Estimate reproductive success of hatchery and natural origin steelhead spawning above the weir in Little Sheep Cr. (Imnaha R.)
7	Evaluate effectiveness of genetic monitoring	a	Quantify genetic differences between hatchery, natural, and wild populations
		b	Quantify sources of noise in analysis (sampling error, genetic drift)
		c	In light of a) and b), evaluate combined power of genetic markers (allozymes + DNA) to provide monitoring and evaluation information that is useful for an adaptive management approach to supplementation.

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	8/89	9/09	Tissue samples logged in the NWFSC collection. 10 years of intensive multibasin genetic monitoring samples. 10 subsequent years of	Each year of successful sampling represents a milestone	10

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
			pedigreed steelhead samples along with abbreviated genetic monitoring samples.		
2	12/89	6/10	Genotypic data obtained for various numbers and types of loci across subsets of samples.	Successful verification of genotypes and standardization with other studies	50
3	3/90	9/10	Comparisons of genetic variability among populations at various spatial and temporal scales.	Publication of results in peer reviewed literature, progress reports, interagency memos	5
4	6/90	9/10	Estimates of N_e/N for hatchery and natural populations.	Tests of various methods published in peer reviewed literature, reports, and memos	5
5	3/90	9/10	Estimates of the hierarchical distribution of genetic variation	Publication of results in peer reviewed literature, reports, and memos	5
6	3/90	9/10	Documentation of particular genetic changes associated with specific artificial propagation programs and a better understanding of the general factors that lead to different outcomes.	Publication of results in peer reviewed literature, reports, and memos	15
7	3/01	3/10	Generate specific guidelines for effective genetic monitoring research with consideration of statistical power and capability to provide practical management information	Published characterization of genetic differences between hatchery and natural populations, examination of	10

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
				accuracy and precision, final presentation of information obtained framed in the context of specific management problems	
				Total	100

Schedule constraints

The major potential constraint in this study is the availability of parr for sampling from wild and natural populations. This was not a problem in 1989-94, but in 1995 and 1996 lethal collections of spring/summer chinook salmon for allozymes were suspended because of record low returns of adults in 1994 and 1995. In those years, we placed more emphasis on nonlethal fin clips for DNA analysis and continued to sample from hatchery populations as feasible. Lethal samples from natural populations in 1997 were restricted to three collections on the South Fork Salmon River. Larger adult returns in 1997 provided for lethal sampling of juveniles from most study sites in the Summer of 1998. Sampling in 1999 should also be feasible without undue risk to wild/natural populations, but the abundance of parr in subsequent years is more uncertain. Appropriate levels, types, and methods of sampling will continue to be determined in consultation with state agency biologists, and through the process of securing state and federal ESA collection permits. Because of uncertainty regarding obtaining lethal samples for allozyme analysis, more of our work in the future will rely on nondestructive DNA analysis. Sample availability will become less constraining as the emphasis of our research shifts increasingly from allozyme to DNA methods in this phase of the study. In this phase of the study, allozyme samples are likely to be taken only every 3-5 years from the wild and natural populations that were formerly sampled approximately every year. The other extreme, that of too many returning adults, could complicate the Little Sheep Creek reproductive success component of the study. Excessive numbers of adults returning to the weir (e.g., 1000 or more) could delay completion of genotyping and increase materials costs (because all potential parents must be typed). Because the samples can be safely stored, some delay in processing them would not seriously compromise the success of the proposed study.

Completion date

This study, which began in late 1989, was designed to run for at least 10 years, or about 3 salmon generations (generation time varies between species and among locations). The technological innovations and basic population genetic data already generated have shown significant utility in management and recovery. Moreover, methodological developments associated with this research have opened new avenues of research not formerly possible (e.g., kinship analysis, pedigree reconstruction, and others). In an effort to address a particularly

acute management question regarding the reproductive success of naturally spawning hatchery fish, we propose to redirect some effort and initiate a new approach to the central research problem. Rather than inferring reproductive success indirectly by measuring allele frequency change through time, we seek to estimate relative success of hatchery and natural fish directly by DNA typing steelhead parents and progeny and reconstructing pedigrees in a natural system above a weir. This research is proposed to continue through 2010 (two steelhead generations to be sampled 2000-2009). Although the frequency of allozyme sampling would be scaled back to accommodate this more intensive analysis, we propose to keep the basic framework of the original experimental design to maintain the continuity of this unique data set. With the same total budget, we will continue traditional genetic monitoring of established study sites (albeit with abbreviated sampling) and also implement this new and very exciting reproductive success component.

Section 5. Budget

FY99 project budget (BPA obligated):	$**225,000**

FY2000 budget by line item

Item	Note	% of total	FY2000 ($)
Personnel		31.4	78.3
Fringe benefits		7.3	18.2
Supplies, materials, non-expendable property		25.3	63.0
Operations & maintenance		2.0	5.0
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		0	0
NEPA costs		0	0
Construction-related support		0	0
PIT tags	# of tags:	0	0
Travel		2.9	7.3
Indirect costs		17.7	44.0
Subcontractor	Field sampling, service, disposal	4.4	11.0
Other	Lab help	9.0	22.5
TOTAL BPA REQUESTED BUDGET			249.3

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
Total project cost (including BPA portion)			

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	$250K	$250K	$250K	$250K

Section 6. References

Watershed?	Reference
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PART II - NARRATIVE

Section 7. Abstract

This genetic monitoring program is designed to evaluate the effects of outplanting hatchery-reared fish on natural and wild populations of spring/summer chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) in the Snake River Basin. The two major goals are 1) to evaluate the nature and extent of genetic changes in hatchery stocks to be used for outplanting, and 2) to quantify the genetic impact of outplanting on targeted natural stocks and non-targeted wild stocks.

Yearly samples are taken from the hatchery stocks used for supplementation and selected natural and wild populations. Samples are analyzed for a suite of protein and DNA characters, and data obtained will be used to test the hypothesis that the outplanted stock has no significant genetic impact on natural or wild populations. Allele frequency change over time will be used to estimate effective population size and rates of inbreeding.

In FY2000, we will begin an intensive and direct evaluation of reproductive success of hatchery and naturally produced steelhead in Little Sheep Creek. Adults of hatchery (marked) and natural (unmarked) origin returning to the weir will be DNA typed using nonlethal fin clips. These data will then be used to establish parentage of juveniles sampled from rearing areas above the weir and subsequent returning adults.

In addition to monitoring the effects of the individual enhancement programs, the study will provide a broader perspective of the kind of results to be expected from different methods of supplementation. Results thus should be of general use in planning and implementing enhancement programs throughout the Columbia River Basin.

Section 8. Project description

a. Technical and/or scientific background

In spite of concerted management efforts, the abundance of most Pacific salmon species (*Oncorhynchus* spp.) has been substantially below historical levels in recent years (e.g., Fraidenburg and Lincoln 1985; Nehlsen et al. 1991; NRC 1995). The Columbia River Basin Fish and Wildlife Program has an interim goal of doubling the abundance of anadromous salmonids in the Columbia River Basin while conserving genetic diversity. An important component of the program is supplementation--that is, the use of artificial propagation to increase the abundance of

naturally-spawning salmon and steelhead (*O. mykiss*). A number of supplementation programs are already under way throughout the basin.

The most recent review of supplementation research (Miller et al. 1990) indicates that there are still substantial gaps in our knowledge of how to supplement natural populations effectively. Among the most important, yet least understood, factors to consider are the genetic consequences of releasing hatchery-reared fish into the wild. This is an important consideration because the genetic makeup of native wild stocks was presumably shaped by hundreds or thousands of years of adaptation to local conditions. Transplanted fish may be less well suited to local conditions, and hybridization may cause a reduction in fitness of the native stock through outbreeding depression. Emlen (1991) reviewed some of the evidence for outbreeding depression in other organisms and suggested a model that may be applicable to Pacific salmon. These possibly adverse effects can be reduced by using a stock for outplanting that is genetically similar to the local stock. However, even a successful supplementation program using local brood stock will entail some genetic change to the local stock. It is important, therefore, to have a means of assessing the nature and extent of genetic changes that occur as a result of supplementation.

Unfortunately, traditional monitoring methods are not well suited to determining whether outplanted fish are having any permanent genetic effect on the target stock. Physical tags may indicate whether a fish returns as an adult, but not whether it produces offspring that survive and contribute to subsequent generations. It is possible, for example, to release large numbers of juvenile fish in a stream over a period of many years and, in the end, not know whether 1) the natural population has been entirely replaced, 2) the current population contains genetic material from both the original population and the outplanted fish, or 3) the outplanted fish have had no permanent genetic impact on the natural population. Hindar et al. (1991) reviewed data from a number of studies of salmonids that show each of these outcomes is possible.

A genetic monitoring program provides the best opportunity for determining which of these scenarios has occurred. Because genetic markers are heritable, they reveal information about the reproductive success of transplanted fish and the degree to which the native and transplanted gene pools have been integrated. Furthermore, the same approach can be used to evaluate the genetic effects of outplants on nearby wild stocks that are not intended to be supplemented.

Recent development of highly polymorphic microsatellite loci now presents a powerful alternative to simply measuring genetic change through time at a population level. It is now possible to establish the parentage of specific individuals in a semi-closed system (e.g., from a finite pool of parents that have been finclipped as they are passed over a weir). For the first time it is possible to obtain a direct "real time" estimate of relative reproductive success of hatchery and natural fish. It will also be possible to examine mate choice and the specifics of gene flow using this approach (with direct estimation of N_e and accurate measure of N).

b. Rationale and significance to Regional Programs

The central tenet of adaptive management, as framed in the 1987 Columbia River Basin Fish and Wildlife Program, is maintaining flexibility to respond to biological indicators of the success or failure of specific management strategies. This flexibility, however, is of little use without an adequate monitoring and evaluation program to provide the basis for making scientifically-based decisions. Supplementation is an experimental strategy that has considerable promise but also many associated uncertainties. The genetic consequences of supplementing natural populations with hatchery reared fish are among the biggest uncertainties, and this issue

cannot be addressed without a monitoring program that focuses on genetic markers. Specifically, reproductive success of naturally-spawning hatchery fish has been repeatedly identified as a critical uncertainty in the appropriate and effective use of artificial propagation in recovery (Grant 1997; NMFS cap on hatchery stray rates; hatchery risk/benefit analyses). This study is thus an essential component of a more comprehensive, cross-disciplinary monitoring and evaluation program for salmon supplementation.

Upriver stocks of chinook salmon and steelhead were given highest priority for research in the FWP. The proposed research directly addresses a number of goals in the revised (1994/95) Program, including 4.1A.2 (which indicates that "program activities should pose no appreciable risk to biological diversity among or within fish populations" and "activities should be followed-up with monitoring and evaluation"); 4.1D (calling for establishment of Biological Diversity Performance Standard that "will be the existing level of biological diversity"); 7.2A.1, parts 1) and 2) (which call for "coordination of hatchery production to reduce [genetic and ecological] impacts of hatchery stock on wild and naturally spawning fish" and "monitoring and evaluation of hatchery and wild and naturally spawning stock interactions"); 7.2A.6 (stating the "goal of increasing sustained production while maintaining genetic resources," "avoiding adverse genetic effects on wild, natural and hatchery fish populations," and "maintenance of genetic integrity (including...effective population size)"); 7.3B.2 (which provides for implementation of high priority supplementation projects, including "design, construction, operation, maintenance, monitoring, and evaluation"); 7.4D.1 (calling for "development of genetically sound methods of sourcing and breeding brood stock to ensure genetic stability"); 7.4L.1 (which directs Bonneville to fund "biological monitoring and evaluation studies" for Northeast Oregon production facilities); 7.5B.1 (which identifies the need to "develop an experimental design for implementing monitoring and evaluating supplementation"); 8.4A.1 (which directs managers to "develop and implement an expanded genetic stock identification program for monitoring inriver and ocean fisheries"); 8.4B.1 (calls for evaluation of "the potential for using DNA 'fingerprinting' and other methods to identify chinook...and steelhead stocks in the Columbia River").

The overall genetic monitoring study design accommodates and addresses a number of issues and recommendations of the Columbia River tribal recovery plan, *Wy Kan Ush Me Wa Kush Wit*. In the Monitoring section of the document a call is made for monitoring of the "effectiveness of individual projects." The studies proposed here involve an annual cycle of sampling, as explicitly recommended in the *Wy Kan Ush Me Wa Kush Wit*, and the reproductive success study attempts to "track life stage survival" among individuals of different parentage. The work proposed here will provide at least two of the important benefits identified in the Implementation and Coordination section of the tribal recovery plan. Benefit #2, the provision of information on rates of natural variation among watersheds, is afforded by the multibasin design of the genetic monitoring research. Both the traditional genetic monitoring research and the reproductive success study make extensive use of existing programs and infrastructure for sample collection. Finally, this proposal takes several of the recommended actions associated with Hypothesis 11, Stock-specific Harvest Management Concerns, including work to "develop assessment methodologies for identifying individual stocks" and to "to track population dynamics" using genetic traits. Another recommended action that is taken in this study is to conduct "genetic monitoring for heterozygosity" at an "escapement checkpoint" (i.e., the weir on Little Sheep Creek). The "least intrusive methods" possible will be used in that most sampling will be done nonlethally by using fin clips.

In addition to addressing the issues above, this study has also provided important

information in a variety of critical areas, including 1) Population structure of Snake River spring/summer chinook salmon for use in listing determinations and recovery planning under the Endangered Species Act; 2) Effects on natural populations of straying by non-native hatchery fish in the Grande Ronde basin; 3) Effective size of wild, natural, and hatchery populations; 4) A temporal series of baseline data for comparison with data from future sampling programs; 5) Population structure of Snake River steelhead for use in the ESA status review for coastwide steelhead populations being conducted by NMFS; 6) Evaluation of potential distinctiveness of populations of particular management interest (e.g., NE Oregon spring chinook salmon captive brood stock collections; marked hatchery adults and unmarked putatively natural fish returning to the Rapid River trap).

Additional benefits anticipated from the Little Sheep Creek reproductive success component of this study include information regarding potential gene flow between resident and anadromous segments of the population. For example, if returning sea-run adults were found to have parents that were sampled as resident fish above the weir, this would provide important documentation of the genetic relationship between resident and sea-run fish. Because of a sampling design that will capture multiple life history stages (age 0-2 parr, adult), we expect that any difference in mortality among classes of offspring (i.e., hatchery x hatchery, hatchery x natural, natural x natural) can be partitioned among life history stages. Finally, establishing parentage for individual juveniles will allow direct measures of mate choice (determining actual gene flow and rate of gene exchange between hatchery and natural components of the population) as well as overall success for hatchery and natural fish as groups.

c. Relationships to other projects

Experimental design for this study was coordinated with state and tribal biologists, and we work closely with these groups each year in planning and conducting sampling. To the extent possible, sampling is also coordinated with other studies to minimize disturbance to the natural populations and maximize usefulness of the genetic and biological information collected. The following are the major related projects we have coordinated with for planning, sampling and/or dissemination of results.

Northeast Oregon: Early life history study of Grande Ronde Basin chinook salmon (Project 92-26-1, ODFW); Smolt migration characteristics and parr-to-smolt survival of naturally produced spring chinook salmon in the Grande Ronde and Imnaha river basins (part of Fish Passage Center smolt monitoring program); Evaluation of reestablishing natural production of spring chinook salmon in Lookingglass Creek, Oregon, using a non-endemic hatchery stock (CTUIR and ODFW, funded through LSRCP); Evaluation of the Lower Snake River Compensation Plan in Oregon (ODFW, funded through LSRCP); Grande Ronde spring chinook captive broodstock collection and maintenance (Projects 9606700, 9604400, and 9801001 funded through ODFW, NPT, LSRCP and NMFS); NE Oregon outplanting facilities plan and hatchery planning (Projects 8805300, 8805301, and 8805305, ODFW and NPT).

Idaho: Performance/stock productivity impacts of hatchery supplementation (Project 90-052, NBS); Idaho natural production and evaluation, Intensive monitoring subproject (Project 91-073, IDFG); Idaho supplementation studies (Project 89-098, IDFG); Monitoring the migrations of wild Snake River spring/summer chinook salmon smolts (Project 91-028, NMFS); Steelhead supplementation studies in Idaho rivers (Project 9005500, IDFG).

In addition, for the past several years we have shared juvenile chinook salmon collected under our study with Drs. Diane Elliot and Ron Pascho of the National Biological Service, who

use the samples for analysis of bacterial kidney disease in their study, "Juvenile fish transportation: Impact of bacterial kidney disease on survival of spring/summer chinook salmon stocks," funded by the US Army COE. This collaboration was temporarily suspended because only fin clips were collected in 1995 and 1996 but may resume if adult returns improve.

d. Project history (for ongoing projects)

This project has retained the same essential focus since it began in 1989. Past costs have averaged $273K per year. Analysis is currently underway for samples from the 10th year of field collections. Although the central focus of the research has remained unchanged, the sampling design has been somewhat flexible to respond to high priority issues associated with supplementation in the Snake River basin that have arisen since 1989. For example, for the past several years we have conducted intensive genetic monitoring in the Grande Ronde basin to assess impacts to natural populations of straying by Rapid River stock hatchery fish. The initial study design identified the only Lostine River as the representative natural/wild population to be sampled in the Grande Ronde basin. Similarly, we have considerably expanded the geographic coverage of our steelhead collections within the basin to evaluate effects of outplanting non-native stocks such as Dworshak and Pahsimeroi.

Major results achieved

Important results obtained to date include the following:

1) Snake River spring/summer chinook have higher levels of genetic variability than had been suggested by previous studies;
2) Snake River steelhead populations are highly polymorphic with a large number of variable loci, which increases power to resolve stock structure and effects of supplementation;
3) Levels of genetic variability in hatchery populations do not differ substantially for those of natural and wild populations;
4) Spring/summer chinook salmon populations are spatially structured and the structure appears to be stable over time. Structure in steelhead appears to be slightly less well-defined, except in the Clearwater basin;
5) The Dworshak Hatchery population of steelhead is the most genetically distinctive population in the Snake River basin. Analysis of natural/wild populations believed to be affected by strays or outplants from Dworshak Hatchery do not show evidence of substantial genetic effects from this stock;
6) The distinctiveness of the Dworshak Hatchery population appears to be a reflection of its ancestry rather than being the result of genetic or demographic events subsequent to domestication;
7) Considerable genetic diversity is found among natural/wild populations of spring/summer chinook salmon from the Grande Ronde basin. However, samples from some streams in some years are genetically very similar to the non-native Lookingglass Hatchery stock;
8) Genetic indices suggest that gene flow among subpopulations is on the order of a few (1-2) migrants per year;
9) Indirect genetic estimates suggest that the ratio N_b/N in natural and wild populations of chinook salmon is about 0.2 - 0.4. These are the most comprehensive data available for any salmon species on this critical ratio;
10) A large number of DNA markers have been developed that greatly increases our ability to monitor supplementation and identify parentage and relatedness;

11) Preliminary work with DNA isolation from archived scale cards showed considerable promise for characterizing historic populations;
12) Nonlethal DNA sampling used to provide genetic information to managers on specific biological problems (e.g., evaluation of distinctiveness of NE Oregon captive broodstock collections relative to the Rapid River stock spawned at Lookingglass Hatchery, and efforts to determine the likely persistence of an endemic gene pool in Rapid River that is distinct from the introduced spring-run hatchery population);
13) Continual technological innovation in both DNA and allozyme methods has substantially increased genotyping efficiency and throughput. DNA now routinely obtained from historic scale samples, and allozyme genotypes (for 30 polymorphic loci) have been obtained nonlethally from frozen fin clips;
14) Microsatellite data collected for Little Sheep Creek steelhead provided input for computer simulations demonstrating the feasibility of identifying parentage in the reproductive success study (a similar study was conducted successfully in our laboratory using wild and captive-reared coho in an artificial spawning channel, L. K. Park unpubl.).

Project Reports and Technical Papers--The following publications were all supported at least in part by funds from this project:

Waples, R. S., D. J. Teel, and P. B. Aebersold. 1991. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual Report of Research to Bonneville Power Administration, Portland, OR, 50p.

Utter, F. M.,R. S. Waples, and D. J. Teel. 1992. Genetic isolation of previously indistinguishable chinook salmon populations of the Snake and Klamath Rivers: Limitations of negative data. Fish. Bull. (U.S.) 90:770-777.

Waples, R. S., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual Report of Research to Bonneville Power Administration, Portland, OR, 179 p.

Park, L. K., P. Moran, and R. S. Waples (editors). 1994. Application of DNA technology to the management of Pacific salmon. Proceedings of the workshop, 22-23 March 1993, Seattle, WA. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Waples, R. S., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1):310-329.

Park, L. K., and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. Reviews in Fish and Fisheries Biology 4:272 299.

Park, L. K., P. Moran, and D. Dightman. 1995. A polymorphism in intron D of the chinook salmon growth hormone 2 gene. Animal Genetics. 2(26):285.

Park, L. K., P. Moran, and D. Nickerson. 1994. Application of the oligonucleotide ligation assay (OLA) to the study of chinook salmon populations from the Snake River. In, L. K. Park, P. Moran and R. S. Waples (eds.). Application of DNA technology to the management of Pacific salmon. U.S. Dep. Commer., NOAA Tech. Memo NMFS NWFSC-17:91-97.

Park, L. K., P. Moran, and D. A. Dightman. 1996. A chinook salmon PCR-RFLP marker in the p53 locus. Animal Genetics 27:127-128.

Moran, P., D. A. Dightman, R. S. Waples, and L. K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhynchus* spp.). Mol. Mar. Biol. Biotechnol. 6:318-330.

Ford, M. J. 1998. Testing models of migration and isolation among populations of chinook salmon (*Oncorhynchus tshawytscha*). Evolution 52:539-557.

Moran, P., D. A. Dightman, L. K. Park. 1998. Nonelectrophoretic genotyping using allele-specific PCR and a dsDNA-specific dye. Biotechniques 24:206-212.

Waples, R. S. 1998. Separating the wheat from the chaff: Spatial and temporal patterns of genetic differentiation in marine species. J. Heredity 89:438-450.

Ford, M. J., P. J. Thornton, and L. K. Park. 1999. Natural selection promotes divergence of transferrin among salmonid species. Molec. Ecol. *accepted.*

Adaptive management implications

As discussed in section 7.c., this monitoring and evaluation research plays an integral role in adaptive management of supplementation within the Columbia River basin. Informed decisions about appropriate management actions cannot be made without detailed information about the effects of actions that have already been taken.

e. Proposal objectives

Objective 1. Collect samples--Based on preseason surveys of juvenile distribution and redd counts from the previous year, collections will be arranged, coordinating wherever possible with other field activities. Samples will include hatchery, natural, and wild collections representing the study sites in different basins.

Assumption:

Sampling is random with respect to the entire population. Again, some departures from strict randomness are expected, but non-representative samples can bias results. In some cases, a sample of progeny from a relatively few individuals can be identified by an unusually low estimated ratio of effective to total population size.

Objective 2. Conduct genetic analyses--Preliminary analyses of allozyme and DNA data will be conducted to assure its integrity and identify any potential errors or sampling anomalies.

Testable hypothesis: Genotypic frequencies do not differ from those expected under Hardy-Weinberg equilibrium

Assumption: Allozyme variation is largely neutral. Undoubtedly some departures from strict neutrality exist, but substantial departures might bias conclusions drawn from the data. With respect to temporal variation, this assumption can be tested as described below under "testable hypotheses."

Objective 3. Measure levels of genetic variability in each population--Genetic variability within populations will be evaluated in a number of different ways. Comparisons of variability in hatchery, natural, and wild populations will be made and changes in levels of variability will be evaluated through time.

Testable hypotheses: 1) Levels of genetic variability are the same in hatchery, natural, and wild populations. 2) Levels of genetic variability do not change over time

Objective 4. Estimate effective population size (N_e) and the ratio N_e/N for each population--Fixation indices and gametic disequilibrium will be used to estimate and evaluate the relationship between effective population size and census size (N) estimated from redd counts, spawner surveys, and population enumeration.

Testable hypotheses: 1) Inter-locus variance of F (a measure of allele frequency change over time) is no larger than would be expected if all changes are due to sampling error and genetic drift. 2) The relationship between N_e and N is the same in hatchery and natural populations. 3) The relationship between wild N_e and N in natural/wild populations is the same in years of high and low escapements.

Objective 5. Evaluate population genetic structure of natural and wild populations--Fixation indices and hierarchical gene diversity analyses will be used to partition genetic variation into spatial and temporal components. These relationships will be used to estimate levels of gene

flow among populations.
Testable hypotheses: 1)There are no genetic differences among natural populations, except those that can be attributed to sampling error and random year-to-year variation. 2) Genetic affinities among geographic populations change randomly over time. 3) Levels of gene flow among populations are less than X individuals per generation.
Objective 6. Evaluate genetic effects of supplementation on target and non-target populations--Indices of genetic differentiation will be calculated between hatchery and natural, and hatchery and wild populations. Patterns of genetic change will be examined through time in the three classes of populations. In the Grande Ronde basin, short-term change over the period of 10 years represented by this study will be compared to change seen since the inception of the Lookingglass Hatchery program. The Little Sheep Creek study presents the opportunity to test specific hypotheses regarding reproductive success, mate choice and concomitant rates of geneflow among hatchery and natural components of the steelhead population.
Testable hypotheses: 1) There are no genetic differences between hatchery populations and natural populations they were derived from. 2) Reproductive success of naturally spawning hatchery steelhead is equal to that of naturally produced fish, and mate choice is random with respect to parentage of individual fish. 3) Populations that have been supplemented show the same magnitude of genetic change over time as unsupplemented populations. 4) Non-target wild populations have not been genetically affected by hatchery strays. 5) Current natural populations in the Grande Ronde basin are not more similar to the Rapid River stock from Lookingglass Hatchery than they were historically. 6) Populations in which genetic effects of supplementation can be detected show the same patterns of abundance and productivity as unsupplemented populations.

Objective 7. Evaluate effectiveness of genetic monitoring--An overall assessment will be made of the power of genetic markers to provide monitoring and evaluation information that is useful for an adaptive management approach to supplementation. We already know that this approach can be very useful in some instances and less useful in others, but we continue to make this evaluation as the data accumulate.
Testable hypothesis: Genetic differences among populations are so small and temporal variation so great that relationships among samples, and effects of supplementation, cannot be meaningfully evaluated. Note: This general hypothesis can be tested for each supplementation program in each of the species (8 tests altogether). If the hypothesis is rejected, then we can evaluate power of the combined genetic data (allozymes + DNA) to detect genetic differences of various magnitudes. Taken as a whole, these results should provide considerable insight into the general usefulness of genetic monitoring and evaluation programs.

f. Methods

Sampling--Yearly samples will be taken from hatchery, wild, and natural populations involved in the study. Initially samples were 100 fish per population per year, but in recent years this has been scaled back to 80, 60, and then 40 fish per sample to minimize effects on at-risk wild and natural populations. Hatchery samples are smolts or presmolts shortly prior to release. Field samples involve parr or smolts; for steelhead, we work closely with local biologists to avoid collecting resident rainbow trout. Field collections are made with seines or electroshockers and are conducted in accordance with NMFS ESA permit #1056, study 2. Allozyme samples are frozen in the field on dry ice or liquid nitrogen and transported or shipped to Seattle for storage and analysis at -80°C. During dissection for allozyme analysis, subsamples of tissue are preserved in ethanol and logged in to the NWFSC tissue collection. More and more of our sampling in the coming years will be taken as nonlethal fin clips from either adults or juveniles. In the case of Little Sheep Creek steelhead samples taken for the reproductive success component of this study, tissue collections will be made nonlethally in the form of fin clips.

Protein electrophoresis--Protein electrophoresis follows the procedures of Aebersold et al. (1987). Laboratory procedures have been standardized among the agencies participating in the Coastwide Genetic Stock Identification Consortium. In particular, we are working closely with Washington Department of Fish and Wildlife personnel to ensure that data gathered by both agencies are compatible and reflect state-of-the-art laboratory techniques.

For each fish, genotypic data will be gathered for a series of enzyme systems coding for approximately 40-60 gene loci known to be variable in chinook salmon (steelhead have comparable, or slightly higher, levels of genetic variability). The number of loci that are polymorphic in any given sample will be fewer and varies somewhat geographically, but typically will be about 20-40.

The protein electrophoretic database for the Snake River genetic monitoring study sites is considerable. Although we anticipate a shift in emphasis from allozymes to nonlethal DNA sampling, we intend to continue with allozyme collections in years when abundance will support lethal sampling. We expect it will be possible to obtain allozyme samples for some of the natural/wild populations at least every 3 to 5 years. Hatchery sampling would remain more regular.

DNA methods--In recent years, the use of DNA techniques has added significantly to the repertoire of research tools available to the salmon genetics community (Park and Moran 1994). DNA markers have served to augment allozyme data, providing additional power to identify subtle differences among populations and small genetic changes through time. They also simplify field collection of tissues, because tissues can be stored and shipped at ambient temperature, rather than requiring dry ice or liquid nitrogen. Further, even small juveniles can be easily sampled nonlethally by taking small fin clips. Most importantly for this work, it is possible to sample historic populations available as archived scale collections.

In this study, two major classes of nuclear DNA markers have been developed and implemented: 1) restriction fragment length polymorphisms (RFLPs) in the introns, 3' untranslated regions (3'UTRs), and other noncoding sequences of nuclear genes; and 2) highly variable simple sequence repeats, or microsatellite loci. Eight RFLP loci and 16 microsatellite loci are fully implemented for chinook; 6 RFLP loci and 9 microsatellites have been implemented in steelhead. An additional nine loci have been optimized individually in steelhead and are ready to implement together as a set. Breeding studies have been completed for many of these loci verifying Mendelian inheritance. Various numbers of DNA loci have been analyzed in particular

populations of interest. The DNA markers showed high levels of variability among populations, and those patterns of variation were broadly concordant with allozymes.

The RFLP methods used in this study follow the approach of Moran et al. (1997) to characterize allele frequency differences among populations. Briefly, salmon nuclear DNA sequences obtained from Genbank or generated in our laboratory are used to design PCR primers that amplify introns or other noncoding sequences (typically 500 - 2000 bp in size). The amplified products are either sequenced or surveyed with restriction enzymes in a subset of individuals to find segregating sites in the populations of interest. Different alleles are represented by the presence or absence of one of more restriction sites. All of the RFLP markers used in this study were developed in our laboratory.

The microsatellite methods are similar to those presented in Olsen et al. (1996). In this case, PCR primers amplify tandem simple-sequence repeats (e.g., the DNA bases CACACA...). Allelic variation is present at these loci in the number of copies of the repeat unit and thus the size of the PCR product. Many microsatellite primer pairs are now available for Pacific salmon. In addition to developing several microsatellite loci of our own, we have taken full advantage of primers available in the research community. We continue to interact quite closely in comparing methods with other salmon research groups including Alaska Department of Fish and Wildlife, Bodega Bay Marine Laboratory, Pacific Biological Station, Washington Department of Fish and Wildlife, University of Idaho, and especially Dr. Paul Bentzen's group at the Marine Molecular Biotechnology Laboratory, University of Washington, School of Fisheries.

During this performance period, our DNA efforts will focus on surveying larger numbers of individuals and populations for the markers we have already developed. We will take advantage of the ability to sample fin clips nonlethally to gather an unbroken temporal series of data from depressed natural populations of spring/summer chinook salmon. We will also attempt to collect historic genetic information from archived scale samples to allow a comparison of genetic profiles pre- and post-supplementation. Preliminary work with chinook salmon scale collections from other regions shows considerable promise for the use of scale archives as a viable approach for characterizing historic populations (manuscript in preparation). These methods should be particularly useful in evaluating the effects of the Rapid River stock hatchery program on natural population structure of chinook salmon in the Grande Ronde basin.

In addition to continuing our surveys for new variable loci, we also intend to devote further effort to developing more rapid methods of conducting DNA assays. We anticipate that our previous work with ligation capture (Park et al. 1994) and allele-specific PCR (Moran et al. 1998) will lead to significantly more efficient assays and will allow examination of base substitutions not associated with restriction site changes.

Data analysis--Electrophoretic phenotypes visualized on starch gels are interpreted as genotypes according to guidelines discussed by Utter et al. (1987). A chi-square test is used to compare genotypic frequencies at each variable locus in each population with frequencies expected under Hardy-Weinberg equilibrium. This test can be useful in detecting artifactual (nongenetic) variation. The method of Waples (1988) is used to evaluate genotypes and estimate allele frequencies at isoloci (duplicated gene loci). A variety of standard statistical analyses are routinely applied to the data (e.g., computing heterozygosity, gene diversity, number of alleles per locus, genetic distances, and *F*-statistics; testing for heterogeneity of allele frequencies among populations).

In addition to these analyses, a number of more specialized analyses are used to estimate

effective population size. As the primary goal of this project is to study genetic changes over time in natural and wild populations resulting from supplementation, it is necessary to consider factors other than hatchery-wild genetic interactions that can lead to genetic change. Because supplementation is typically considered only when natural abundance is low, the effects of random genetic drift due to finite population size must be considered in evaluating observed genetic changes. Our methods for estimating effective population size include the following:

1) Quantifying allele frequency change. The statistic used to measure the magnitude of genetic change is $F = (P_1 - P_2)^2/[P(1-P)]$, where P_1 and P_2 are allele frequencies in samples taken at two different times and P is the mean of P_1 and P_2. F is computed for each gene locus surveyed, and a mean F over all loci in a comparison of temporally spaced samples is also computed.

2) Testing for selection. Although there is a body of evidence suggesting that the enzymatic gene loci sampled by electrophoresis in general are largely unaffected by natural selection, it is important to evaluate this assumption because strong selection would complicate the interpretation of changes within populations and interactions between populations. If the loci used are effectively neutral, they all should be affected by genetic drift to approximately the same degree. The method of Lewontin and Krakauer (1973) will be used to test the hypothesis that the variance of single locus F values is no larger than expected from random sampling error.

3) Measuring gametic disequilibrium. The statistic r^2, the squared correlation of alleles at different gene loci, are computed for each pair of loci in each sample. The overall mean r^2 value is a measure of gametic disequilibrium, or non-random associations across loci.

4) Estimating N_b. After omitting any loci identified by the test for selection, the mean F value (computed as in #1) is used to estimate N_b, the effective number of breeders each year. The procedure follows the "temporal method" for estimating effective population size (Krimbas and Tsakas 1971; Nei and Tajima 1981; Waples 1989), as modified specifically for Pacific salmon (Waples 1990).

Because F is known to be distributed approximately as chi-square, confidence limits can be placed on the estimate of N_b. The mean value of r^2 provides an independent method for estimating N_b, based on the method developed by Hill (1981), and confidence limits can also be placed on this estimate.

Reproductive success of hatchery and naturally produced steelhead adults spawning naturally. Fin clips will be taken from all adults passed over the weir on Little Sheep Creek. Resulting juveniles will be sampled in rearing areas above the weir and assigned to specific matings based on comparison of multilocus genotypes among candidate parents. This will be achieved by using both exclusionary criteria (finding a parent/mating that result in an exact match to a particular juvenile, (e.g., Pedigree, C. Busak, Washington Department of Fish and Wildlife; Comparez 5.0, J. B. Taggert, Queens University), and probabilistic approaches that explore the likelihood of each possible parentage assignment and establish statistical criteria for accepting the true parent (Cervus 1.0, Marshall et al. 1998). Each year, samples of 250-500 juvenile fin clips will be taken from selected sites at various distances upriver from the weir. Juvenile sampling will be done twice over the summer field season (providing temporally spaced replicates), carried out under contract to Nez Perce Tribal and ODFW field biologists. Once the matings are assigned for a sample of offspring, it will be possible to evaluate the proportion of matings (i.e., hatchery x hatchery, hatchery x natural, and natural x natural) relative to the expected proportions based on the numbers of hatchery and natural fish above the weir.

Simulations have been conducted using allele frequency data from Little Sheep Creek steelhead (BY 1991) for a suite of highly polymorphic microsatellite loci. Results demonstrated that genotyping errors and missing parents can combine to significantly diminish the power of parentage analysis. However, discussions with state and tribal biologists indicate that the weir on Little Sheep Creek is essentially impassible to both adults and juveniles moving upstream, and there are likely to be very few missing parents for juveniles sampled above the weir.

Regarding error rates, we found in another parentage study conducted in an artificial spawning channel that errors were quite low (typically less than 0.5%), and when they occur can usually be isolated and corrected, resulting in a confident parentage assignment (L. Park, NW Fisheries Science Center, unpubl.). Further, error rates can be mitigated by analyzing more loci or more polymorphic loci. The we propose to run a suite of 18 microsatellite loci that will provide enormous power to resolve parentage and, based on simulation results, should more than compensate for any uncertainty introduced by genotyping error.

Evaluating genetic effects on natural/wild populations. Several different methods can be employed in this evaluation, depending on the type of data available. The most important question is whether pre-supplementation baseline data are available for the hatchery and natural/wild stocks involved.

a) Baseline data available. In the short term (up to about 1 generation after supplementation), the proportion of fish of hatchery and wild origin can be estimated using the mixture model of Milner et al. (1981). A variety of methods can be used to place confidence limits on the estimated contributions. In the longer term, the relative contribution of two original gene pools to a hybridized mixture can be estimated using the method discussed by Glass and Li (1953). This approach can be modified to take genetic drift into consideration (Thompson 1973).

b) Baseline data not available. Power to resolve the genetic contribution of hatchery and natural fish is reduced considerably if pre-supplementation baseline data are not available. However, the null hypothesis that the existing population represents a single gene pool (rather than a mixture of gene pools) can still be tested using gametic disequilibrium analysis. Gametic disequilibria are correlations of alleles at different gene loci, and one cause of these disequilibria is a mixture of different gene pools. Waples and Smouse (1990) showed that the power to detect mixtures of salmonid populations can be reasonably high provided that there were sufficiently large genetic differences between the stocks before mixing. This method, however, has limited power to detect mixtures involving populations that are genetically similar.

g. Facilities and equipment

Conservation Biology's Genetics Program--the oldest fishery genetics program in the country--is well equipped and staffed to carry out the research proposed here. Since its inception in the late 1960's, the Genetics Program has played a central role in the development of Pacific salmon genetics research on the West Coast. In cooperation with other agencies, the Genetics Program has helped build coast-wide genetic data bases for all the North American Pacific salmon species. In addition to embracing the commonly used methods of population genetic research in salmon, the Genetics Program has pioneered new methods and new classes of markers in both DNA and allozyme analysis. With six PhD-level geneticists working on a wide range of research problems and a first-rate technical staff, this group has the experience, training, creativity and vision to take on large-scale long-term projects such as the genetic monitoring research proposed here. The Genetics Program is in a unique position to conduct the new reproductive success study because of the technological capability that has been obtained over the past 8 years in the development and

implementation of powerful new genetic methods for Snake River chinook salmon and steelhead.

Protein Genetics Laboratory
The protein genetics laboratory is a newly remodeled, fully equipped, state of the art facility for allozyme population genetic data collection. The laboratory is equipped with 15 power supplies to meet a demanding gel running schedule, a fume hood and balance weigh station for the proper use and handling of hazardous chemicals, two personal computers for recording the allozyme genetic data and for keeping track of the samples being processed, a digital camera for recording the genetic results, as well as a number of standard laboratory items such as balances, refrigerator, freezer, centrifuge, dish washer, pH meter, and water bath. In addition, the laboratory has seven ultra cold freezers for proper storage of the samples to be analyzed. The laboratory is staffed by three full time technicians and a laboratory manager.

DNA Laboratory
The DNA laboratory is a fully equipped, state-of-the-art facility for molecular genetic R&D and population genetic data collection. In addition to standard molecular genetic laboratory equipment (pipettes, pan balances, pH meter, microcentrifuges, conventional and ultra-cold freezers, and PC and Macintosh computers), the following instrumentation and resources (with the exception of the UV plate reader) are available for our exclusive use (i.e., not a shared core facility): Two automated fluorescent DNA sequencers, ultraviolet/visual plate reader, seven thermal cyclers, High-speed refrigerated tabletop centrifuge (for isolating DNA from tissue samples in a 96-well format), DNA analysis software (including automated genotyping), custom horizontal electrophoresis chambers (12-channel, 96-sample format), and vertical sequencing chambers (also 12-channel format).

h. Budget

Personnel--covers two co-PIs time for project coordination, oversight and management, data analysis, and report preparation; technical staff to perform laboratory allozyme and DNA analyses.

Fringe benefits--22% of personnel costs for vacation, sick leave, etc.

Supplies--Laboratory chemicals, disposable plasticware, and other consumable supplies for DNA and allozyme laboratories (including dry ice, ethanol, express shipping and other costs associated with sample collections. Costs for this item are slightly ($14.5K) higher than in the previous year, reflecting more intensive DNA genotyping of Little Sheep Creek steelhead.

Operations and maintenance--$5K for laboratory utilities

Travel--costs include staff time and a vehicle for field collections, and travel to regional meetings for coordination, planning, and reporting results.

Indirect costs--standard rates for NOAA overhead based on labor costs

Subcontractors--$7K for contracts with ODFW and NPT to take fin clips of adults at Little Sheep Creek weir and make field collections of juveniles; $22.5K for laboratory staff for allozyme and DNA analyses; $4K for laboratory service contracts and hazardous waste disposal.

Total costs--this is the same total level this project has been funded at for the past 5 years, although in FY99 all projects in the group were reduced by 10% (to $225K).

Section 9. Key personnel

Robin S. Waples, Acting Director, Conservation Biology Division

B.A. in American Studies, Yale University, 1969.

Ph.D. in Marine Biology, Scripps Institution of Oceanography, 1986.
1986-present, research geneticist or fishery biologist at the Northwest Fisheries Science Center in Seattle

FTE commitment for FY2000 = 0.15

Dr. Waples developed this study and has been principal investigator since its inception. He has six years of experience in field collections of chinook salmon and steelhead for this study and is familiar with all the geographic areas and most of the local biologists. His research background is in the population genetics of fishes, and he has published widely on topics such as the analysis of temporal genetic changes, hatchery-wild genetic interactions, genetic methods for estimating effective population size, and identification of conservation units for salmon under the ESA.

Many of the statistical analyses of the genetic data use techniques he developed several years ago for the study of temporal genetic variation in Pacific salmon.

Five relevant publications:

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379-391.

Waples, R. S., and D. J. Teel. 1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conserv. Biol. 4:144-156.

Matthews, G. M., and R. S. Waples. 1991. Status review for Snake River spring and summer chinook salmon. U.S. Dep. Commer., NOAA Tech. Memo. NMFS F/NWC-200, 75 p.

Waples, R. S., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1):310-329.

Allendorf, F. W., and R. S. Waples. 1996. Conservation and genetics of salmonid fishes. Pages 238-280 in: J. C. Avise and J. L. Hamrick, eds. Conservation genetics: Case histories from nature. Chapman and Hall, New York.

Paul B. Aebersold, Biological Technician

A.A.A. (1977) Marine Biology Technology, Shoreline Community College. National Marine Fisheries Service, Conservation Biology Division (1978 - present). Manager of the protein electrophoresis laboratory, collects genetic data and prepares for final analysis.

FTE commitment for FY2000 = 0.4

Paul Aebersold has worked as a Biological Technician for the National Marine Fisheries Service for almost 20 years. His responsibilities include overseeing and supervising the daily operation of the laboratory; scheduling the laboratory work to be done; interpreting the electrophoretic results; confirming the data being collected; preparing the data for statistical analyses; training new laboratory technicians or visiting scientists in protein electrophoresis techniques and in the interpretation of the results; assisting in the writing of reports and/or manuscripts; and leading the research effort in pursuing new techniques and new technologies which may improve our ability to meet our objectives. Mr. Aebersold also has over 10 years experience in the use of microcomputers to manage large genetic datasets, as well as 5 years of experience in basic network administration.

Five relevant publications:

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. U.S. Department of Commerce, NOAA Technical Report NMFS 61, 19 p.

Waples, R. S., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Report to Bonneville Power Administration, Contract No. DE-AI79-89BP00911.

Winans, G. A., P. B. Aebersold, and R. S. Waples. 1996. Allozyme variability of *Oncorhynchus nerka* in the Pacific northwest, with special consideration to populations of Redfish Lake, Idaho. Transactions of the American Fisheries Society 125:645-663.

Waples, R. S., P. B. Aebersold, and G. A. Winans. 1997. Population genetic structure and life history variability in *Oncorhynchus nerka* from the Snake River Basin. Report to Bonneville Power Administration, Contract No. DE-A179-93BP05326.

Winans, G. A., P. B. Aebersold, Y. Ishida, and S. Urawa. In press. Estimates of stock composition of chum salmon in highseas test fisheries in the western north Pacific Ocean and Bering Sea. Proceedings of the North Pacific Anadromous Fish Commission (NPAFC), October, 1996. Sapporo, Japan.

Gregory Mackey, Research Molecular Geneticist

B.A., in Biology, University of Southern Maine (1995)

M.S., candidate, School of Fisheries, University of Washington (1998)

National Marine Fisheries Service, NW Fisheries Science Center, Conservation Biology, Genetics, (Sept.1998 - present)

FTE commitment for FY2000 = 0.62

Greg Mackey is responsible for DNA data collection, sample tracking, and molecular genetic R&D associated with this project. He has four years of molecular genetics experience, including RFLP analysis and multiplex analysis of microsatellite markers using a fluorescence-based DNA sequencer. In addition to his thesis research on reproductive success of steelhead in a Puget Sound stream (via parentage analysis), Mr. Mackey has conducted studies on molecular and quantitative genetics of fruit flies (*Drosophila melanogaster*), benthic macroinvertebrate ecological indices, *E. coli* monitoring for stream water quality, and salt marsh ecology. He has extensive experience sampling adult and juvenile salmonids at a smolt fence and adult weir, and using electrofishing and seining. He has also conducted two seasons of steelhead radio-tracking and collected fecundity samples from hatchery spawning operations. Other experience includes SQL programming to build a database that identifies parent and offspring matches based on multilocus genotype, database management of ecological and genetic data, and supervising student interns and field crews.

Relevant publications:

Mackey, G., Rhodes, J., and T. P. Quinn. 1996. Steelhead reproductive success and coho salmon hatchery-wild competition in Forks Creek I. Progress report to Weyerhaeuser Company. Includes review of data collected and preliminary analysis.

Mackey, G., Rhodes, J., and T. P. Quinn. 1997. Steelhead reproductive success and coho salmon hatchery-wild competition in Forks Creek II. Progress report to Weyerhaeuser Company. Includes review of data collected and preliminary analysis.

Paul Moran, Research Population Molecular Geneticist

B.S., in Biology, Southern Oregon State College (1985)

M.S., in Biology, Central Washington University (1988)

Ph.D., in Zoology, University of Maine (1993)

National Marine Fisheries Service, NW Fisheries Science Center, Conservation Biology, Genetics, (1992 - present)

FTE commitment for FY2000 = 0.46

Dr. Moran directs laboratory research in the development of DNA markers and population genetic data collection and analysis. Research interests and activities include molecular phylogeography, population genetics, and changes in the genetic structure of populations over various temporal scales. Duties include supervision of students and technicians, as well as safety and environmental compliance responsibilities for the laboratory. Dr. Moran has over 12 years of experience in molecular genetics research, including evolutionary biology, systematics and population genetics. He is competent with commonly used computer programs for analysis of population genetic data (e.g., Arlequin, Biosys, Cervus, GDA, Genepop, NTSYS, Phylip, Popgene, REAP, TFPGA, Winamova). For the past 6 years, Dr. Moran has been involved in the development of PCR-RFLP and microsatellite markers in Pacific salmon. He has also developed allele-specific PCR and ligation capture assays. He is trained in hazardous materials emergency response, laboratory safety, environmental compliance, and hazardous materials shipping regulations. Dr. Moran also has considerable experience in field collection including PIT-tagging of salmonids, snorkeling, electrofishing, and seine netting.

Five relevant publications:

Moran, P. 1994. Overview of commonly used DNA techniques. In, L. K. Park, P. Moran, and R. S. Waples (eds.), Applications of DNA technology to the management of Pacific salmon, p. 15-26. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Moran, P. and E. Bermingham. 1994. The phylogeographic structure of coho salmon (*Oncorhynchus kisutch*) populations assessed by mitochondrial DNA. Abstract in, L. K. Park, P. Moran, and R. S. Waples (eds.), Applications of DNA technology to the management of Pacific salmon, 57-62. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFS-17, 178 p.

Park, L. K. and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. In, G. R. Carvalho and T. J. Pitcher (eds.), Molecular genetics in fisheries, Reviews in Fish Biology and Fisheries. 4:272-299.

Moran, P., D. A. Dightman, R. S. Waples, and L. K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhynchus* spp.). Mol. Mar. Biol. Biotechnol. 6:318-330.

Moran, P., D. A. Dightman, L. K. Park. 1998. Nonelectrophoretic genotyping using allele-specific PCR and a dsDNA-specific dye. Biotechniques 24:206-212.

Section 10. Information/technology transfer

We will continue to use a variety of methods to disseminate results of this research. Examples of reports and journal articles resulting from previous years of this project are listed elsewhere in this document. In 1993 we used funding from this project to host a workshop on application of DNA

technology to the management of Pacific salmon and to publish the proceedings. A subsequent DNA workshop was hosted in 1997. Results of various aspects of this study have been presented in numerous public meetings throughout the region as well as nationally and internationally. In addition, we have provided many informal summaries of recent or unpublished results to fishery and hatchery managers in Washington, Idaho, and Oregon. These results have been used in an adaptive management framework to make real-time decisions about issues such as broodstock collection, mating protocols, and release strategies. Our efforts to develop RFLP loci are somewhat unique in salmon genetics and this work is beginning to bear fruit both within the Columbia River and elsewhere. We have distributed PCR primers to many research groups in North America, Asia, and Europe, including groups funded by BPA (e.g., Matt Powell's group at University of Idaho).

Congratulations!